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Specification and Drawings, as originally filed, with Application for Patent Serial No:  
2,273,576, on May 27, 1999, by **CECIL YIP, PETER OTTENSMEYER AND  
ROBERT Z-T LUO**, for "Three-Dimensional Quaternary Structure of the Insulin  
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**Three-dimensional quaternary structure of the insulin receptor****(Abstract)**

The 3D structure of the intrinsically dimeric insulin receptor (IR) bound to its ligand, insulin, was determined by electron cryomicroscopy. Insulin labeled with gold served to locate the insulin-binding domain on the 3D structure. The 3D structure was then fitted with available known high resolution domain substructures to obtain a detailed contiguous model for this heterotetrameric  $(\alpha\beta)_2$  transmembrane receptor. The 3D reconstruction shows that the two  $\alpha$  subunits jointly participate in insulin binding and that the two  $\beta$ -subunit kinases in this receptor-ligand complex are in a juxtaposition that permits *trans*-autophosphorylation of key tyrosine residues in the first step of insulin receptor activation.

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Large transmembrane proteins such as cell surface hormone receptors have been difficult to crystallize as intact molecules for high-resolution structural study. They are also too large for NMR spectroscopy. The 480-kDa insulin receptor (IR) has thus not been crystallized as an intact molecule, and its quaternary structure remains unknown. In this study we have taken an alternative approach to study the quaternary structure of IR: low-dose low-temperature dark field scanning transmission electron microscopy (STEM). Using electron micrographs of the insulin-IR complex we have reconstructed the three-dimensional quaternary structure of the intact receptor complexed with gold-labeled insulin ligand. Although IR has been purified and studied for over 15 years, this is the first 3D reconstruction of its entire dimeric structure. Contiguous high densities within the 3D structure indicate a two-fold symmetry for this dimeric membrane receptor, as well as a logical sequence for its biochemical subdomains from the observed binding of a single insulin on the ectodomain to the juxtaposition of the pair of intrinsic tyrosine kinases (TKs) of the intracellular domain.

Insulin binding to the cell surface insulin receptor is essential for its manifold effects such as glucose homeostasis, increased protein synthesis, growth, and development in mammals. IR belongs to the superfamily of transmembrane receptor TKs that include the monomeric epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR). In contrast, IR and its homologues IGF-I R (insulin-like growth factor I) and IRR (the insulin receptor-related receptor) are sub-types of this family that are intrinsic disulfide-linked dimers of two heterodimers of the form  $(\alpha\beta)_2$  (1,2). Monomeric receptor TKs are inactive, but are activated by ligand-induced dimerization that results in

autophosphorylation. Dimeric IR-like TKs are also inactive, and are activated by ligand binding without further dimerization. Insulin binding to the extracellular domain of IR results in autophosphorylation of specific tyrosines in the cytoplasmic domain to initiate an intracellular signal transduction cascade (3). However, the structural basis for the mechanism of IR activation by extracellular insulin binding has not been elucidated because the quaternary structure of IR is not known. Only some of the smaller domains have yielded high resolution structural information. The crystal structure of its intracellular TK domain has been solved, providing important insights into the molecular basis for its activation (4). In addition, the crystal structure of the first three N-terminal domains (L1-Cys-rich-L2) of the highly analogous IGF-I R has been solved (5). Although without binding activity, this monomeric fragment showed a structural template for binding. In the case of IR, the purified dimeric ectodomain, whose structure not yet solved but which contains two L1-Cys-rich-L2 domains, binds insulin with the same affinity as the intact IR (6). Here we report the 3D reconstruction of the quaternary structure of the intact IR bound to insulin marked with Nanogold. The 70-atom gold marker localized and delimited the insulin-binding site on the 3D reconstruction. Its location was key in the determination of structural relationships of other IR domains in the 3D reconstruction of IR which provide a structural basis for IR activation by insulin. In the absence of ATP which is required to complete the activation of the IR tyrosine kinase, the structure of this insulin-bound IR can be considered to be in a transitional state, with its kinase domains intermediate between the inactive and activated structures observed by x-ray crystallography (4).

Compared to native bovine insulin, Nanogold-bovine-insulin (NG-BI), derivatized at the B-chain Phe1(7), a location not directly involved in receptor binding (8), bound to human insulin receptor (HIR) with only a slightly reduced affinity (Fig. 1). Purified solubilized HIR used in this study has been shown to be fully active (9). Such HIR, incubated with NG-BI to form the HIR/NG-BI complex in the absence of ATP (10), was subjected to low-dose dark field STEM imaging at  $-150^{\circ}\text{C}$  (11). Figure 2A shows a representative field of individual molecules. On average, each HIR/NG-BI complex measured 15 nm across. Based on its strong scattering, the 1.4 nm gold ligand of NG-BI was located on the image directly as a clear site of highest density, or could be demonstrated as such by thresholding. Figure 2B shows examples of molecules with 1 or 2 sites of highest density, indicative of binding of one or occasionally two NG-BI particles, consistent with the known binding of between one and two insulins per IR (3,12). When two NG-BI particles were detected, they were in close proximity to each other.

Approximately 700 images were selected for reconstruction on the basis of having a definite site of high density, the expected mass for the complex, being structurally contiguous, and being separated from neighbouring images (13). The 3D reconstructions of the HIR/NG-BI complex are shown in Figure 3. The interpreted alignment and the fit of the biochemical domains to this structure are detailed in Fig. 4. The 3D structure at the full expected volume is compact and globular (Fig. 3A, top panel). The NG-BI particle was located on the 3D reconstruction by increasing the density threshold without imposing symmetry (Fig. 3A, panel 2 and 3), to pinpoint the binding site and to limit the fit of insulin

to its vicinity within the IR complex. Since insulin binds to the L1-Cys-rich-L2 regions of the ectodomain of IR (14), the NG cluster identifies this region of IR in the reconstruction.

Domain-like features of the structure become evident at intermediate density thresholds (Figure 3A, panel 2), and, except for the NG-BI region, these indicate a strong 2-fold vertical rotational symmetry as anticipated from the dimeric configuration of the oligotetrameric  $(\alpha\beta)_2$  structure of IR. This symmetry was used to reduce noise in the reconstructions and render the structures shown in panel 1 and in Figure 3B, putatively as being viewed in the plane of the membrane, and in the extracellular (top) and intracellular (bottom) perspectives. Views of these structures are reminiscent of the X- and Y-shaped electron microscopic images previously observed for IR or its ectodomain (15).

In the side views, the top part of the structure, where NG is located, is identified as the ectodomain of the  $\alpha$  subunit. The dog-bone-shaped substructure of the 3D reconstruction, (Fig. 3B, top view), and equivalently the top-most, bow-tie-shaped structure (Fig. 3B,  $0^\circ$ ), are designated as the two L1 domains of the dimeric receptor on the basis of the x-ray structure of the L1-Cys-rich-L2 domains. The side view at  $65^\circ$  shows the putative L1-Cys-rich-L2 domains as contiguous substructures across the upper central region of the molecule, with enough additional volume in this region to account for most of the remaining mass of the two  $\alpha$  subunits, primarily the connecting domains (CD).

The contiguity of the domain structure (Fig. 3B, top and side view  $90^\circ$ ), along with the primary domain sequence (Fig. 4A), shows that the two  $\beta$  subunits occupy the lower half of the structure, distal from L1, reaching up and out as a contiguous mass. The intracellular TK domain of IR would then occupy the bottom portion of this structure with

two IR fibronectin type III (FnIII) repeats in each receptor half (16) being in the top portion of the crescent-shaped spiral of the  $\beta$  subunit at the same level as the L2 domain in the  $\alpha$  subunit. One of the FnIII repeats, composed of residues from both the  $\alpha$  and  $\beta$  subunit, is assigned to the upper left end of the crescent (side view, 0°) where it is contiguous with the CD portion of the  $\alpha$  subunit (top view). It has been suggested recently that the CD has a fibronectin-like structure (17). Fig. 4C and 4D (cf. Fig. 3B, 90°, top view, respectively) show the fitting of the crystal structure of the TK domain (green) of the  $\beta$  subunit and of the two FnIII repeats (blue/red) modelled as the canonical fibronectin type III structures (16).

The masses of the putative kinase domains are connected via a slender horizontal bridge (Fig. 3B, side view 90°) that was not observed in the x-ray structures of the TKs, but can be explained in terms of the reconstruction being in a transition between free IR and its ligand-activated form. In the two symmetrically fitted TK (Fig. 4C and 4D) crystal structures the catalytic loops are separated by 4 nm. This distance is just sufficient to permit the tyrosine triplet (Tyr1158, 1162 and 1163) in a fully extended flexible activation loop of one TK to reach the catalytic loop of the opposite TK as modelled from the x-ray coordinates (PDB 1IRp). The extension of the activation loops, equivalent in cross-section to four extended polypeptide chains, easily accounts for the linking density observed between the lower portions of the  $\beta$  subunits (Fig. 3B, 90°). This is an important difference from the x-ray structures of the inactive and activated TKs as discussed below.

The spatial relationship between the domains of the  $\alpha$  and  $\beta$  subunits (e.g. side view, 90°) shows the location of the cell membrane lipid bilayer as the space below the  $\alpha$  subunits and above the bridge linking the two assigned TK domains. Instead of a flat open

region, this space in the 3D reconstruction forms a thick dome-like slab above the bridge with a thickness variation of 2.2 to 2.7 nm. This spacing is a change in shape from, and a decrease in the thickness expected for a membrane bilayer that would accommodate an alpha-helical transmembrane domain (TM) of 23-26 hydrophobic amino acids. However, since the purified IR in the absence of its native membrane was fully active, the relative positions of the extracellular and intracellular domains must still represent a close to native arrangement.

The crossing L1-Cys-rich-L2 domains of the dimeric  $\alpha$  subunits are presented here only in their general shape (Fig. 4B and 4C), since the known x-ray coordinates have not yet been made available (5). Nonetheless, using this structure, the localization of the gold cluster, and the known receptor-binding domain of insulin (8), we have tentatively fitted an NG-BI molecule into this region. The best fit is obtained with a molecule of insulin, partially on the two-fold symmetry axis of the dimer, being in contact with the L1-Cys-rich domains of one  $\alpha$  subunit and with the L2 domain of the other  $\alpha$  subunit. A model involving both  $\alpha$  subunits in the high-affinity binding of insulin has previously been proposed based on studies of insulin analogues binding to IR and IR/IGF-I R chimeras (18). Our 3D reconstruction provides the structural evidence for this involvement. Although two molecules of insulin can be fitted to this configuration, two molecules of Nanogold-labeled insulin were observed only rarely in the STEM images. The high-affinity binding of the first insulin molecule to the IR has induced a conformational change in the binding domain so that the second insulin molecule would bind only at low affinity. Likewise the binding of a second molecule of insulin could



effect a conformational change that enhances the dissociation of the bound insulin. Thus the curvilinear Scatchard plot and the negative cooperativity of insulin binding (19) can be explained on the basis of the 3D reconstruction. The reconstruction also explains why only low-affinity binding is obtained with purified  $\alpha\beta$  monomer (20).

Superimposition of known crystal structures of smaller domains of the receptor on substructures of the 3D reconstruction has made it possible to deduce the spatial relationship among the domains in the complex. The structure shows the division of the complex into the extracellular and the cytoplasmic segments along a plane, the putative cell membrane, on which the fibronectin type III repeats lie (16-18). These repeats appear pontoon-like to support the centrally located insulin-binding segment of the ectodomain.

Monomeric inactive receptor TKs such as EGFR are brought together by ligand binding and become activated as dimers resulting in TK autophosphorylation (21). In the intrinsically dimeric IR-family receptors, the distance between the two cytoplasmic  $\beta$ -subunit TKs within the dimer must be too great without ligand binding for the activation of the kinase. Hubbard *et al.* (4) suggested that insulin binding to IR decreased this distance by disengaging Tyr1162 from the catalytic loop to enable *trans* phosphorylation in the presence of ATP. In our reconstruction a good fit to the ligand-receptor complex is obtained when the two TK domains are oriented with their catalytic loops juxtaposed. In this orientation the extended flexible activation loop of each TK, which moves 30 Å between the inactive and activated states in the crystal structures (4), can just reach the catalytic loop of the opposing TK to be activated. These two loops can easily form the linking mass density between the TKs seen in the 3D reconstruction in the absence of ATP.

The 3D structure obtained from images of the HIR-complex containing only a single NG-BI, shows that one molecule of insulin is sufficient to bring the two  $\alpha\beta$  monomers to an activating configuration. The dimeric receptor with a Ser323Leu mutation in the L2 domain of both  $\alpha$  subunits showed a severe impairment in insulin binding (22), whereas a hybrid receptor with only one of the two  $\alpha$  subunits mutated was found to bind insulin with high affinity and was fully active as a tyrosine kinase (23). Based on our 3D reconstruction, insulin bound to the L1 domain of the mutant  $\alpha$  subunit and the wild-type L2 domain of the hybrid IR and the binding of only a single molecule of insulin is sufficient for TK activation.

In this study we have obtained the 3D quaternary structure of the IR-insulin complex formed in the absence of ATP. The structure was an intermediate between insulin-free IR and the fully activated, phosphorylated IR. The reconstruction is readily interpreted as such: as a receptor poised for activation by *trans*-phosphorylation. We determine the full extent of conformational changes induced by insulin binding. We reconstruct the initial state of free IR and the final activated state for comparison. We obtain a crystallographic structure of the insulin receptor. The 3D reconstruction presented here provides concrete structural information towards the full understanding of transmembrane signal transmission in insulin action. Furthermore, the approach used in this study can be applied to obtain the quaternary structure of other membrane proteins or receptors that are refractory to crystallization. The invention includes the methods for studying polypeptide structure described in this application.

## Figure Legends

Preferred embodiments are described in relation to the drawings, in which:

**{PRIVATE }Figure 1.** Receptor-binding assay of Nanogold-insulin. Receptor-binding activity of purified Nanogold-insulin was compared to that of bovine insulin in a receptor-binding assay using human insulin receptor as described (9). Inset shows the mass spectrum obtained from the MOLDI-TOF analysis of purified Nanogold-insulin (7). {tc  
**"Figure 1.** Receptor-binding assay of Nanogold-insulin. Receptor-binding activity of purified Nanogold-insulin was compared to that of bovine insulin in a receptor-binding assay using human insulin receptor as described<sup>21</sup>. Inset shows the mass spectrum obtained from the MOLDI-TOF analysis of purified Nanogold-insulin. "

**{PRIVATE }Figure 2.** STEM dark field images of human insulin receptor /Nanogold-insulin (HIR/NG-BI) complex. A) Raw images showing several complexes. Arrowheads point to intense signals from Nanogold marker. Scale bar = 20 nm. B) HIR/NG-BI images extracted from image fields, after low pass filtering to 1.0 nm and boundary determination (left column). High density threshold representation of extracted images showing one (top five images) or two (bottom two images) sites of Nanogold location (right column).{tc  
**"Figure 2.** STEM dark field images of human insulin receptor (HIR)/Nanogold-insulin (NG-BI) complex. a) Raw images showing several complexes. Arrow heads point to obvious intense signal from Nanogold marker. Scale bar = 20 nm. b) HIR/NG-BI images extracted from image fields, after low pass filtering to 1.0 nm and boundary determination (left column). High density threshold representation of extracted images showing one (top five images) or two (bottom two images) sites of Nanogold location (right column)."

**Figure 3.** Three-dimensional reconstruction of the HIR/NG-BI complex from 704 STEM dark field images. A) Density threshold representing the total expected volume for the complex [1]; intermediate density threshold, unsymmetrized, showing higher contiguous densities [2]; high density threshold of [2] showing only the Nanogold label [3]. Circles in the panels indicate location of the gold marker within the reconstructions. The resolution was 20 Å as measured by Fourier phase residual analysis of two reconstructions with 352 images each (13). B) Reconstruction with two-fold symmetry at intermediate density thresholds in different orientations, indicating the relationship and connectivity of the structural domains. Labels, for only one  $\alpha\beta$  monomer of the dimeric HIR, refer to biochemical domains. Arrowhead indicates the proposed plane of the cell membrane lipid bilayer. L1, C-R, L2 = L1-Cysteine-rich-L2 domains; CD = connecting domain; Fn1, Fn2 = fibronectin III repeats 1 and 2; TK = tyrosine kinase; TM = transmembrane domain.

**Figure 4.** Fitting of biochemical domains and their known x-ray structures to the 3D reconstruction. A) Schematic domain structure for one  $\alpha\beta$  monomer, derived from i) connectivity of the 3D reconstruction at intermediate density threshold (Fig. 3), ii) from the primary domain sequence, iii) from the requirement for two disulfides on the two-fold symmetry axis between the two  $\alpha$  subunits (24), iv) the fit of the known domain structures, and v) the principle of keeping domains of unknown structure as compact as possible. Distances measured in the 3D reconstruction between locations of subdomains CD, Fn1 and the symmetrical disulfides were commensurate with numbers of intervening amino acid residues (structures not shown to scale;  $\alpha$  subunit shown in red,  $\beta$  subunit in blue and green, unknown structures are spheres or lines): A = TK activation loop; 1 = Cys524; 2 = Cys682,

683, 685; 3 = alpha-beta disulfide between Cys647 and Cys872; arrowhead = proreceptor cleavage site; other labels as described in Fig. 3B. B) Representative fitting of L1-Cys-rich-L2 domains as approximate cylinders to ectodomain structure of 3D reconstruction (cf. Fig. 3B, side view, 0 ). One insulin molecule (purple ribbon, PDB: 1BEN) inserted with its receptor-binding domain contacting the L1-Cys-rich domains of one subunit (fuchsia) and the L2 domain of the other (red). The Nanogold marker (yellow) on Phe1 of insulin B chain positioned to coincide with the high-density site of reconstruction. C) Right angle side view of (B) (cf. Fig. 3B, side view 90 ) with L1-Cys-rich-L2 domains (insulin partly hidden), fitted TK structure (green ribbon, PDB: 1IRK) and two dimeric FnIII structures (blue and red ribbons, PDB: 1mFn). Red ribbons represent the portion of Fn1 derived from subunit. Activation loop (black ribbon) of left TK domain is shown in its crystallographic position. A-loop (dark blue) of symmetry-related right TK domain extended to overlap peptide substrate position of opposite TK in peptide-bound state (4). See also (D). D) Right angle top view of (B) (cf. Fig. 3B, top view) showing the positions of the FnIII domains (top and bottom, blue/red) and the TK domains (green). Crystallographic position of activation loop (black) is uppermost within one TK domain, while extended activation loop (dark blue) of the other TK domain is below. One square in the wire mesh is 6.5 Å.

The present invention relates to insulin receptor (DNA and amino acid sequence is known and available in, for example, US 4,761,371), the structure of insulin receptor as determined by STEM, the use of that structure to solve the structure of insulin receptor homologues and other forms of insulin receptor, mutants and co-complexes of insulin receptor, and the use of the insulin receptor structure and that of its homologues, mutants, and co-complexes to design modulators of insulin receptor or insulin receptor interactions or activity when the receptor is associated with insulin. The structure is useful for development of ingestible (preferably oral) insulin mimicking agents (mimetics) that can be used in place of insulin (which has to be administered by injection) to treat insulin-dependent diabetes.

Those of skill in the art recognize that a variety of techniques are available for constructing polypeptide mimetics with the same or similar desired biological activity insulin but with more favorable activity than the polypeptide with respect to route of administration, solubility, stability, and/or susceptibility to hydrolysis and proteolysis. See, for example, Morgan and Gainor, *Ann. Rep. Med. Chem.*, 24:243-252 (1989). Examples of polypeptide mimetics are described in U.S. Patent Nos. 5,643,873. Other patents describing how to make and use mimetics include, for example in, 5,786,322, 5,767,075, 5,763,571, 5,753,226, 5,683,983, 5,677,280, 5,672,584, 5,668,110, 5,654,276, 5,643,873. Mimetics may be designed on computer by comparing compounds to the 3D structures disclosed in this application. Mimetics of insulin may also be made according to other techniques known in the art. For example, by treating a polypeptide of the invention with an agent that chemically alters a side group by converting a hydrogen group to another group such as a hydroxy or amino group. Mimetics preferably include sequences that are either entirely

made of amino acids or sequences that are hybrids including amino acids and modified amino acids or other organic molecules.

In one aspect the present invention is directed to the three-dimensional structure of an isolated and purified polypeptide designated insulin receptor and its structure coordinates. Another aspect of the invention is to use the structure coordinates of the insulin receptor crystal to reveal the atomic details of the active site and one or more of the accessory binding sites of insulin receptor. The entire receptor may be characterized or particular regions of interest may be characterized. Structural and conformational changes induced in the receptor may also be studied. Another aspect of the invention is to use the structure coordinates of an insulin receptor to solve the structure of a different insulin receptor or a mutant, homologue or co-complex of insulin receptor. A further aspect of the invention is to provide insulin receptor mutants characterized by one or more different properties compared to wild-type insulin receptor. Another aspect of this invention is to use the structure coordinates and atomic details of insulin receptors or mutants or homologues or co-complexes thereof to design, evaluate (preferably computationally), synthesize and use modulators of insulin receptor that prevent or treat the undesirable physical and pharmacological properties of inadequately or improperly functioning insulin receptor. These modulators may be used as therapies that are beneficial in the treatment of diabetes and other diseases, disorders and abnormal physical states characterized by improper or inadequate insulin receptor.

Modulators may be combined in pharmaceutical compositions according to known techniques. The compounds of this invention are preferably incorporated into pharmaceutical dosage forms suitable for the desired administration route such as tablets, dragees, capsules, granules, suppositories, solutions, suspensions and lyophilized compositions to be diluted to obtain injectable liquids. The dosage forms are prepared by conventional techniques and in addition to the compounds of this invention could contain solid or liquid inert diluents and carriers and pharmaceutically useful additives such as

liposomes, aggregants, disaggregants, salts for regulating the osmotic pressure, buffers, sweeteners and colouring agents. Slow release pharmaceutical forms for oral use may be prepared according to conventional techniques.

Pharmaceutical compositions used to treat patients having diseases, disorders or abnormal physical states could include a compound of the invention and an acceptable vehicle or excipient (Remington's Pharmaceutical Sciences 18<sup>th</sup> ed, (1990, Mack Publishing Company) and subsequent editions). Vehicles include saline and D5W (5% dextrose and water). Excipients include additives such as a buffer, solubilizer, suspending agent, emulsifying agent, viscosity controlling agent, flavor, lactose filler, antioxidant, preservative or dye. The compound may be formulated in solid or semisolid form, for example pills, tablets, creams, ointments, powders, emulsions, gelatin capsules, capsules, suppositories, gels or membranes. Routes of administration include oral, topical, rectal, parenteral (injectable), local, inhalant and epidural administration. The compositions of the invention may also be conjugated to transport molecules to facilitate transport of the molecules. The methods for the preparation of pharmaceutically acceptable compositions which can be administered to patients are known in the art.

The pharmaceutical compositions can be administered to humans or animals. Dosages to be administered depend on individual patient condition, indication of the drug, physical and chemical stability of the drug, toxicity, the desired effect and on the chosen route of administration (Robert Rakel, ed., Conn's Current Therapy (1995, W.B. Saunders Company, USA)).

The insulin receptor described in this application was extracted from human placenta. Insulin from other sources, such as other tissues, cells or cDNA may also be used. The techniques described in this application to image the receptor may be used with insulin receptor from any human, mammalian or other tissue. Insulin receptor homologues and other forms of insulin receptor, mutants and co-complexes of insulin receptor may also be used. A fragment of the receptor may also be used. A fragment



may be from about 25-50, about 50-100, about 100-250 or about 250-500 or greater than about 500 amino acids.

Techniques described in this application and known in the art (such as those in references 4 and 5 or US 5,834,228) may be used to identify and characterize other regions of an insulin receptor such as the LI-Cys-rich-L2 domain. We characterize the entire insulin receptor and its active site using these techniques.

The 3D structure may be used in drug design and for screening compounds that may modulate (preferably agonize or antagonize) receptor activity. Drug design may be based on the whole insulin receptor structure or parts thereof (suitable parts of receptor are described in this application). A known in vitro or in vivo assay may be used to further evaluate or test the compound's effects on interaction or activity (eg by measuring in vivo or in vitro binding of receptor to insulin after addition of a compound).

The present invention has been described in detail and with particular reference to the preferred embodiments; however, it will be understood by one having ordinary skill in the art that changes can be made thereto without departing from the spirit and scope thereof.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety

We claim:

1. A method of identifying a compound that modulates insulin receptor activity, comprising:
  - a) designing a compound for modulating insulin receptor activity based upon the 3D structure of insulin receptor;
  - b) synthesizing the compound;
  - c) determining whether the potential compound modulates the activity of the insulin receptor.
2. A method of identifying a compound that modulates insulin receptor activity, comprising:
  - a) comparing a compound for modulating insulin receptor activity to the 3D structure of insulin receptor to determine whether the compound is likely to modulate insulin receptor activity;
  - b) determining whether the potential compound modulates the activity of the insulin receptor.
3. A method of identifying a compound that modulates insulin receptor and insulin interactions or activity, comprising:
  - a) designing a compound for modulating insulin receptor activity based upon the 3D structure of insulin receptor bound to insulin;
  - b) synthesizing the compound;
  - c) determining whether the potential compound modulates the interactions or activity of the insulin receptor and insulin.
4. A method of identifying a compound that modulates insulin receptor and insulin interactions or activity, comprising:

- a) comparing a compound for modulating insulin receptor activity to the 3D structure of insulin receptor bound to insulin to determine whether the compound is likely to modulate insulin receptor and insulin interactions or activity;
  - b) determining whether the potential compound modulates the interactions or activity of the insulin receptor and insulin.
5. The method of claims 1-4, comprising drug design.
6. The method of claim 1 or 2, wherein the compound agonizes the activity of the insulin receptor.
7. The method of claim 1 or 2, wherein the compound antagonizes the activity of the insulin receptor.
8. The method of claim 3 or 4, wherein the compound agonizes insulin receptor and insulin interactions or activity
9. The method of claim 3 or 4, wherein the compound antagonizes insulin receptor and insulin interactions or activity
10. The method of any of claims 1-9, wherein the compound is designed or compared based upon the 3D structural coordinates of insulin receptor.
11. The method of claim 10, wherein the compound is designed or compared based upon the 3D structural coordinates of insulin receptor bound to insulin.
12. The method of any of claims 1-11, further comprising designing or comparing a compound based upon the 3D structure coordinates as described in this application.

13. The method of claim 12, wherein the 3D structure coordinates are obtained by electron microscopy.
14. The method of claim 13, wherein the electron microscopy comprises STEM.
15. The method of any of claims 1-14, wherein the insulin receptor comprises intact insulin receptor.
16. The method of any of claims 3 to 15, wherein the insulin receptor is bound to insulin.
17. The method of any of claims 1-16 further comprising determining whether the potential compound modulates the activity of the insulin receptor or insulin receptor-insulin interactions or activity by an in vitro or in vivo assay.
18. The method of any of claims 1 to 17, wherein the compound comprises an insulin mimetic.

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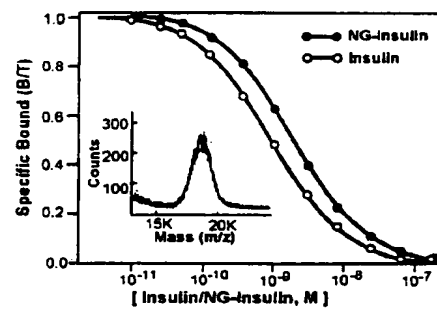


Fig. 2. Lew et al

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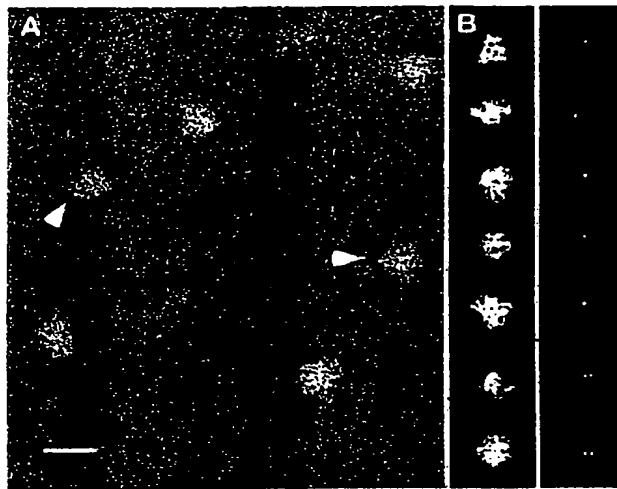


Fig. 2 Luo et al

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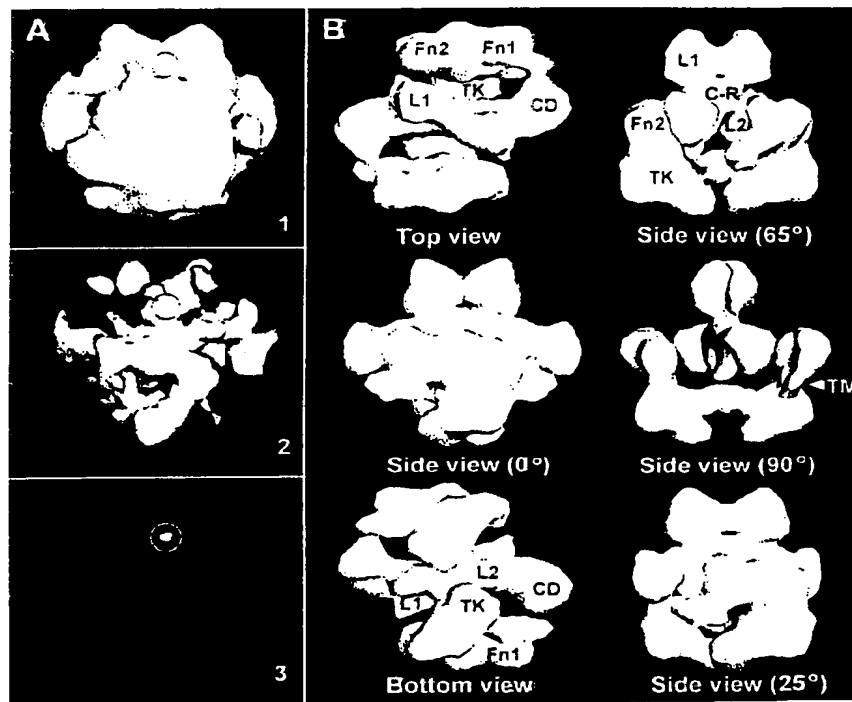


Fig. 3 Luo et al.

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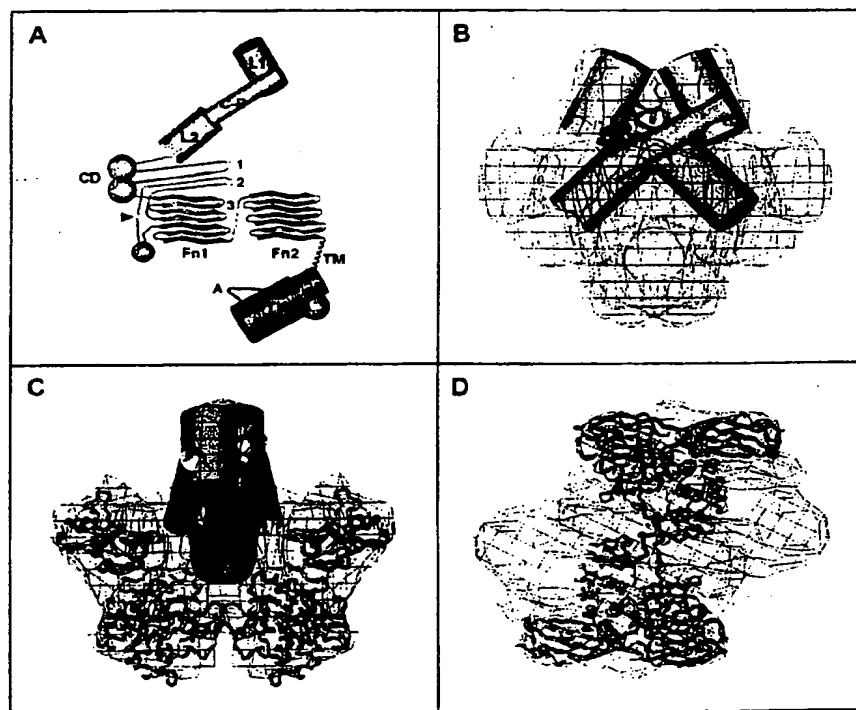


Fig. 4 Luo et al